Alopecia Areata and Universalis in the Smyth Chicken Model for Spontaneous Autoimmune Vitiligo

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The Smyth line (SL) chicken model for spontaneous, postnatal expression of vitiligo may also show varying incidences and degrees of severity ranging from alopecia areata-like to universalis-like integumental changes. Although human vitiligo patients are known to have a four times greater chance of having alopecia areata than do people without vitiligo, in the SL model, feather loss is limited to birds that show some degree of amelanosis of feather and skin tissue. Both the vitiligo and the alopecia have an autoimmune component, as shown by histologic and immunologic studies, including the correctional influences of corticosterone and cyclosporine-A. The major histocompatibility haplotype (MHC) has a major effect on the incidence and expression of the vitiligo, as well as the alopecia that occurs within vitiliginous birds. Three different MHC haplotypes were identified in the original line that was selected for vitiligo, and from these, three sublines were developed, each homozygous for a different haplotype. Of the three sublines (SL101, SL102, and SL103) the vitiligo has a significantly earlier onset and severity in the SL101 than in the other two lines. The incidence of alopecia, however, is significantly lower in the SL101 subline than in the other two. Inheritance of the vitiligo is polygenic with an additional genetic component for the alopecia trait. It is hypothesized, but as yet unproven, that a feather development defect interacts with the SL melanization and immunologic defects to initiate the partial (areata) and complete (universalis) alopecias. The alopecia universalis is rarely seen until adulthood and is characterized by short (<0.5 cm), undeveloped feathers. If feather growth resumes in these birds, the feathers dry up, cease to grow, and often break off. ***Key words: animal model/abnormal feathering/heritable autoimmune defect/dinitrochlorobenzene/triamcinolone acetonide/fluocinonide. Journal of Investigative Dermatology Symposium Proceedings 4:211–215, 1999***

A feathering defect resembling human alopecia areata or alopecia universalis is sometimes expressed in the Smyth line (SL) chicken model for spontaneous autoimmune vitiligo (Smyth *et al*, 1981a; Smyth, 1989). Because the alopecia has only been observed in SL birds that show vitiliginous losses of melanin in their feathers and choroids, a brief description of the SL line is in order (see Boissy and Lamoreux, 1988, and Smyth, 1989, for reviews). The vitiliginous SL strain was originally referred to as the DAM chicken (for delayed amelanotic) and publications through 1985 used this name; however, Dr. Aaron B. Lerner (1984) proposed that the line be renamed the Smyth chicken, which has been the name in common usage since 1985–86.

The vitiligo characteristic of the SL line was first observed in 1971 in a single female from an established line with brown feather color referred to as the Brown line (BL). The BL is considered the SL parental line and at present shows a 1%–2% incidence of a mild spontaneous vitiligo in each generation. The incidence of amelanosis in the SL line increased to 75%–90% after four generations of individual selection for vitiligo. The degree of pigment loss is variable, with many birds eventually developing totally white plumage, although a few may continue to produce both amelanotic and pigment feather tissue simultaneously into adulthood. Pigment loss in developing feathers may start anytime between 4 and 30 wk of age. Some birds remelanize after losing their ability to deposit feather melanin, and may or may not re-enter a vitiliginous phase (Smyth *et al*, 1985; Smyth, 1989). During the early development of the SL, several other associated traits were identified, including a severe retinal dystrophy leading to blindness, a hypothyroidism, and an alopecia areata-like feathering defect (see Smyth, 1989, for review). The blindness was found in approximately 40% of amelanotic birds (Smyth *et al*, 1981b) and was found to be related to the severity of the inflammatory processes associated with melanocyte destruction in the choroid. The hypothyroidism was found to be genetically very similar to that of the OS chicken model for Hashimoto's thyroiditis, an autoimmune disease (see Jerszyk, 1983, and Wick, 1987, for reviews). The first defective feathering was observed on a female of the second generation of selection and she was totally denuded by 1 y of age. That this form of alopecia was due to an autoimmune reaction was shown by Jerszyk (1983).

Evidence for an autoimmune component in the expression of the vitiligo includes: (i) histologic observations at both the light and the electron microscopic level of developing feather and choroidal tissue (Smyth *et al*, 1981a, b; Boissy *et al*, 1983; Erf *et al*, 1995); (ii) major role of the major histocompatibility complex (MHC) (for review, see Smyth, 1989); (iii) successful treatment by corticosterone (Boyle *et al*, 1987) and cyclosporine-A (Pardue *et al*, 1987); (iv) delayed expression of vitiligo following neonatal bursectomy

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Figure 1. A 14-wk-old male from the SL 103 subline showing the basic feathering defect that precedes the varying degrees of alopecic feather loss.

Figure 2. One-year-old SL male and female exhibiting severe alopecia areata. These birds will be completely denuded following their next feather molt.

(Lamont and Smyth, 1981); (v) autoantibodies directed specifically towards melanocytes (Austin *et al*, 1992; Austin and Boissy, 1995); and (vi) association with other autoimmune defects, Hashimoto's thyroiditis, and alopecia-like defective feathering (see Smyth, 1989, for review).

It is our hypothesis that the vitiligo phenotype does not occur unless an inherent melanocyte defect is present (Smyth, 1989). Surgical bursectomy altered the expression of the vitiligo (Lamont and Smyth, 1981) but did not alter the melanosome abnormality (Boissy *et al*, 1984). SL melanocytes have also been shown to display their abnormalities in culture in the absence of possible influences by the immune system (Boissy *et al*, 1986). The relative roles of documented immune responses, e.g., autoantibodies, melanocyte autophagocytosis (Boissy *et al*, 1983), or altered T cell subset expressions in the feather pulp (Erf *et al*, 1995) are not clear at this time. Other influences, including the effects of 5-Azacytidine on gene methylation in the SL amelanosis (Sreekumar *et al*, 1996) and the roles of endogenous viruses[1] on the incidence and expression of vitiligo in the SL model, are unclear at present, but interesting.

DESCRIPTION OF THE ALOPECIA-LIKE FEATHERING DEFECT IN SL CHICKENS

The original observations of the alopecia-like feathering defect were of birds with the most extreme, or universalis, form. Subsequent observations showed that the alopecia is not apparent in the down plumage of newly hatched chicks. The earliest expression is a raggedness of the main flight feathers of birds 8–18 wk old (Jerszyk, 1983; Smyth, 1989). The body plumage also becomes ragged (**Fig 1**) and consists of many poorly developed short pin feathers (sheathed, 1–2 cm in length), which leads to the appearance of patchy baldness (areata) in some areas. This condition may persist indefinitely, or eventually after 10–12 mo of age, lead to severe denudation (**Fig 2**). Attempts to refeather occur in older birds, but newly emerging feathers are again abnormal, appear to dry up, and break off.

Alopecic SL chicks do not grow as well as their normally feathered sibs and require supplemental heat to compensate for body heat loss due to poor, or absent, feather cover. Even the most severely denuded alopecic birds can be maintained under heat lamps indefinitely, although they have a low reproductive rate, even when artificial insemination is practiced.

[1]Lakshmanan NK, Smyth JR, Jr., Ponce de Leon FA: Incidence of endogenous viral loci (ev) in Smyth line chickens: an avian model for autoimmune vitiligo. *Poultry Sci* 71 (Suppl 1):90, 1992 (abstr.)

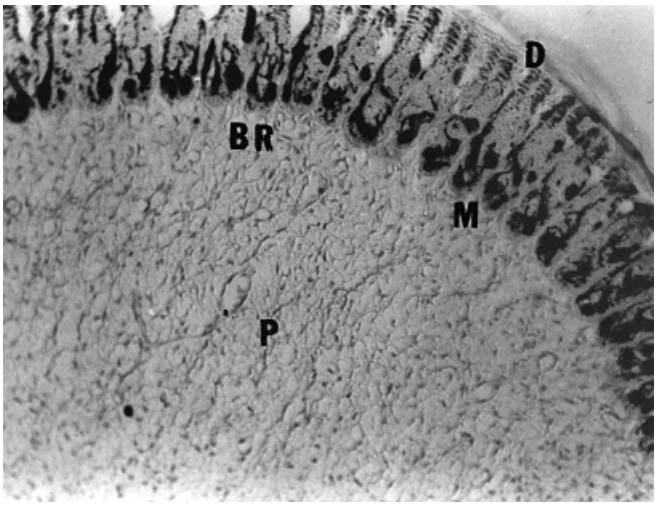

Figure 3. Cross-section through a developing normal feather in barb ridge (BR) area. Note absence of leukocytes in the pulp (P) and the melanocytes (M) in the barb ridges depositing melanin into the developing barb cells (D), 292×.

The development of a feather is a more complex process than is the development of a hair (see Lucas and Stettenheim, 1972, for review). The feather's structure develops on the periphery of the follicular cone and is supported prior to keratinization by the pulp that is vascularized and normally devoid of leukocytes (**Fig 3**). In contrast, the pulp from 80% of the developing alopecic feathers shows an abnormal amount of cellularity as shown by light microscopy (**Fig 4**). The cellularity is due to the invasion of a mixture of large and small lymphocytes and other leukocytes, including eosinophylls (based on paraffin embedded tissue, sectioned at 4–6 microns and stained with standard hematoxylin and eosin). Punch biopsies of the skin of alopecic birds also showed the presence of lymphocytes in two of seven birds (Jerszyk, 1983); however, such evidence of dermal inflammation was not necessarily proximal to developing alopecic feathers.

ASSOCIATION WITH THE MHC

As mentioned previously, the incidence and expression of the SL defects – vitiligo, retinal dystrophy, and the alopecia-like feathering

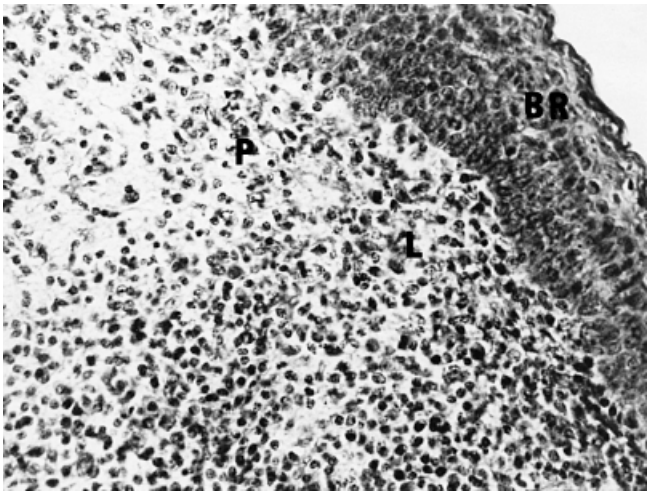

Figure 4. Cross-section through a developing alopecic feather. Cellularity (L) in the pulp (P) and dying melanocytes in the barb ridges (BR) are shown, 292×.

defect – were found to differ significantly between the MHC haplotypes segregating in the original SL populations (see Smyth, 1989, for review). Three segregating haplotypes, B101, B102, and B103, were identified. Because of the associations of the SL characteristics with the MHC, SL sublines, each homozygous for one of the haplotypes, were developed by selective breeding (Smyth, 1989). In addition, three MHC-matched parental control sublines (BL) were also selected to serve as controls for MHC effects in future studies (**Table I**). The formation of these lines was completed in 1992 after three generations of selection and testing for haplotype homozygosity. The incidences of the SL-associated defects (vitiligo, blindness, alopecia-like feathering defect, and hypothyroidism) are summarized for subline generations 1, 3, and 6 in **Table II**. Because there was no consistent difference between the sexes in these three generations, sex data were pooled to simplify the comparisons in the table. The differences in the incidence of vitiligo between sublines are considered to be due to sampling variations; however, note the major subline differences for the incidences of the alopecia and blindness. Surprisingly, although the alopecia appears to be expressed only in vitiliginous birds, it does not usually occur in the SL101 subline that has the earliest onset and most extreme expression of the pigment defect (onset data not shown). The retinal dystrophy was found to be highly associated with the early, more severe inflammation observed at the time of the autoimmune destruction of choroidal melanocytes (Smyth *et al*, 1981b; Boissy *et al*, 1983). It is possible that the absence of the alopecia in the SL101 line may also be related to the later onset of the pigment cell defect. In general, the differences in SL-related characteristics in the SL101, SL102, and SL103 sublines, mirror the relationships seen earlier between MHC haplotype and SL characteristics in the original base SL population (see Smyth, 1989, for review).

Although the association of the autoimmune elimination of functioning melanocytes in the developing feather and the keratinization of the feather structure shown above is interesting, the basis for this relationship is unclear. The subline data suggest involvement of the MHC, presumably mediated through specific immune responses; however, it should be noted that a relationship between these developmental processes supersedes the MHC effect in the keratinization of the rachis of SL feathers as shown at the point of change from distal melanin deposition to a proximal amelanotic stage. At this point in the development of a feather, a detectable circular enlargement of the rachis can readily be felt by sliding one's thumb and index finger along the rachis. This suggests some effect of the altered pigmentation on keratin structure. This appears in mature body feathers regardless of alopecia areata status.

Table I. Vitiliginous Smyth (SL) sublines each homozygous for a different MHC haplotype and their nonvitiliginous MHC-matched parental controls (BL)

MHC-matched SL subline	Homozygous MHC haplotype	MHC-matched parental control
SL 101	B101	BL101
SL 102	B102	BL102
SL 103	B103	BL103

Table II. Three generation phenotypic incidences of defects in SL chicken sublines SL101, SL102 and SL103, each homozygous for a different MHC haplotype[a]

Generation and subline	Number of birds	Vitiligo %	Blindness %[b]	Alopecia %[b]	Hypothyroidal %[c]
1st Generation					
SL101	86	80.2	53.6	0.0	0.0
SL102	54	92.7	0.0	9.8	3.9
SL103	100	83.0	4.8	12.1	0.0
3rd Generation					
SL101	131	89.1	42.0	0.0	2.2
SL102	120	92.5	0.0	8.1	0.8
SL103	123	94.3	8.6	21.6	8.9
6th Generation					
SL101	111	95.5	21.7	0.0	2.2
SL102	76	94.7	0.0	16.7	5.3
SL103	62	90.3	4.8	32.1	3.2

[a]Haplotypes include B101, B102, and B103.
[b]Percentage incidence of vitiliginous birds in subclass.
[c]Percentage incidence of hypothyroidism in all birds in subclass.

INHERITANCE OF SL AUTOIMMUNE ALOPECIA

In addition to the association with the MHC and the expression of vitiligo in the SL, there is additional genetic variation associated with the development of alopecia areata and/or universalis. Furthermore, it appears that a basic feather abnormality appears to be necessary before progression to the expression of alopecia. Although genetic components involved in the above prerequisite phenotypes should also be included in the alopecic genotype, it is those alopecia-inducing genes that we know little about and in which we are most interested. A selection experiment conducted in our laboratories showed that the incidence of alopecia could be significantly increased by individual phenotypic selection of severely alopecic birds as breeders (Jerszyk, 1983). In two selection generations, the incidence of alopecic birds was increased from 0.6% in a base population to 17.4%. This suggests relatively few genes and additive gene action, as does the unintentional increase in the incidence of alopecia in the SL102 and SL103 (**Table II**). In the absence of a definitive genetic analysis of each of the contributing characteristics culminating in the alopecic phenotype, it will have to suffice to consider inheritance as polygenic or familial.

RESPONSE OF ALOPECIC BIRDS TO CORTICOSTEROID TREATMENT

Because corticosteroid administration has been used successfully to stimulate regrowth of hair in human alopecia areata patients (Damon *et al*, 1978; Unger and Schemmer, 1978), several such treatments were tested on alopecic SL chickens to study their effects on the expression of the SL-related defect. The treatments used in our lab included corticosteroid-like substances Kenalog-10 (triamcinolone acetonide) and Lidex (fluocinonide), as well as dinitrochlorobenzene (DNCB).

Five severely alopecic females from the F_2 generation of the alopecia-selected line were treated topically with Lidex (fluocinonide, 0.05% concentration). Applications were made on the right thigh once a day for an 8 wk period, repeatedly treating the same totally unfeathered area. The only effect during the treatment period was to cause a swelling of the follicular collars of the skin. No changes were observed for an 8 wk period following cessation of treatment.

Kenalog-10 (triamcinolone acetonide, 10 mg per ml) was used to treat five severely alopecic females by IM injection. Birds were injected intramuscularly once at two sites, 1 inch apart, with 0.05 cc of Kenalog to result in a concentration of 1 mg per bird. Four of the five birds failed to survive for 10 d postinjection. Feather regrowth and repigmentation occurred in the surviving bird 8 wk following treatment. At this time histologic examination of newly developed feathers and their melanocytes showed them to appear normal with no evidence of inflammatory cells in the pulp. Several weeks later, the amelanosis and alopecia returned. Kenalog-10 was also used in another experiment where the main objective was to observe changes in pigmentation following intradermal injections at a range of dosages designed to resemble those tolerated by humans (JJ Nordlund, Department of Dermatology, University of Cincinnati Medical School, Cincinnati, OH, personal communication). In this case, in two experiments each involving 10 treated birds, none survived for 48 h post-treatment (Smyth, unpublished data).

It has been known for some time that dinitrochlorobenzene (DNCB) can be an effective treatment for alopecia areata in man (Damon *et al*, 1978; Happle *et al*, 1978; Van Neste *et al*, 1980). Presumably, skin-surface application of DNCB elicits a contact allergy response at the affected site, stimulating an eventual influx of suppressor T cells that can dampen the primary immune response against some target antigen on the hair follicle along with that induced by the DNCB treatment. Less is known about the specifics of this reaction in chickens, but increases in dermal populations of polymorphonuclear granulocytes and some monocytes were observed at 24 h post-challenge, 20%–30% of the granulocytes being eosinophiles (Awadhiya *et al*, 1982).

Currently, diphencyprone (DPCP), a similar acting pharmaceutical with fewer side-effects, has replaced DNCB as the treatment of choice for chronic severe alopecia areata.[2] Although the mechanisms of the two are not completely understood, both can be categorized as topical immunotherapeutic agents.

In our study on the effects of DNCB on the SL alopecia areata, five birds with varying degrees of feather abnormalities were sensitized by skin applications of DNCB (0.1% solution) on the right upper thigh once per week for 8 wk. Feathering abnormalities for these birds varied from alopecia universalis to severe, alopecic areata-like feathering defects. No dermatitis was observed on the treated skin surfaces for any of the treated birds. Five SL birds with severe feathering defects, but no alopecia, were used as uninjected controls. Six weeks following treatment a noticeable increase in body feathering was noted for two birds that had been totally denuded. The feathering occurred sparsely but evenly over the whole body. An additional bird that was described originally as being 50% alopecic showed a marked degree of improved feather coverage. The remaining two birds that had been in a pin-feathered stage at onset of treatment also showed marked feather development. A number of the newly grown feathers showed normal pigmentation. The untreated birds showed no increase in feather development and actually demonstrated a slight deterioration in feather cover, so the treatment with DNCB was responsible for the refeathering response in the treated SL birds.

A change in the cellular infiltrate present in the pulp of the regenerating pinfeathers was detected in five of the six samples examined histologically. Functional melanocytes were producing pigment granules that were distributed sparsely into the keratinocytes. The cellular infiltrate was composed mainly of small lymphocytes and dense conglomerates of heterophils. The usual heavy population of eosinophils was not observed in the regenerating feathers. Moderate lymphocytic and/or eosinophilic infiltration was observed in three of the four nonpigmented pinfeathers examined from these birds. This did not appear to differ from that described in feather pulp from untreated alopecic birds.

[2]Shapiro J, Sundberg JP, McElwee KJ, *et al*: Immunophenotyic profiles during topical immunotherapy of alopecia areata in mice and rats with diphencyprone. Speaker synopsis, Third International Research Workshop on Alopecia Areata, Washington, D.C., 11/5/98.

SUMMARY

The main objective of this presentation is to acquaint dermatologists with the Smyth chicken line, a promising animal model for spontaneous alopecia areata and universalis. Similarities between the disease in humans and the SL model include the involvement of autoimmunity with the target cells being present in the developing integument, probably the keratinocytes. The MHC plays a major role in both the incidence and the expression of the disease, whereas other genes appear to have an additive genetic effect on the final phenotype. The remedial effects of certain corticosteroids are also similar to those seen for human alopecia patients. One difference between the human and avian alopecias is the closeness of the association between alopecia areata/universalis and vitiligo. In the chicken model, alopecia areata occurs only in vitiliginous birds, and although there is a definite relationship in the human disease, the association is not as close. Interestingly, however, the SL101 subline that has the earliest onset and most severe vitiligo does not exhibit alopecia areata.

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